



**EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2016.
Scientific opinion on the safety of fermented soybean extract NSK-SD® as a novel food
pursuant to Regulation (EC) No 258/97**

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Safety of fermented soybean extract NSK-SD[®] as a novel food pursuant to Regulation (EC) No 258/97

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)

Abstract

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the fermented soybean extract NSK-SD[®] as a novel food (NF) submitted pursuant to Regulation (EC) No 258/97 of the European Parliament and of the Council, taking into account the comments and objections of a scientific nature raised by Member States. The NF is the fermented soybean extract NSK-SD[®], which is standardised to a nattokinase enzyme activity of 20,000–28,000 fibrin degradation units/g. The information provided on the composition of the NF, the specifications, batch-to-batch variability and the stability is sufficient and does not raise safety concerns. The proposed maximum intake is 100 mg NSK-SD[®]/day as a food supplement. The target population proposed by the applicant is healthy men and women over the age of 35 years, excluding pregnant and lactating women. The Panel noted that nattokinase exhibits *in vitro* fibrinolytic activity and *in vivo* thrombolytic activity in animals when administered parenterally. However, the information provided with respect to absorption, distribution, metabolism and excretion of the NF does not allow conclusions to be drawn on the absorption of active nattokinase or any functional metabolites therefrom. A bacterial reverse mutation test did not show any indication of mutagenicity, and the NF was not clastogenic in an *in vitro* chromosome aberration assay. Taking into account the no observed adverse effect level (NOAEL) of 1,000 mg/kg body weight per day in the subchronic toxicity study in rats, and considering the proposed maximum intake level for the NF, the Panel concludes that the margin of exposure is sufficient. The Panel concludes that the NF, the fermented soybean extract NSK-SD[®], is safe under the intended conditions of use as specified by the applicant.

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Keywords: fermented soybean extract, nattokinase, NSK-SD[®], novel food, safety

Requestor: European Commission following an application by Japan Bio Science Laboratory (JBSL)

Question number: EFSA-Q-2015-00294

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Summary

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the fermented soybean extract NSK-SD® as a novel food (NF) submitted pursuant to Regulation (EC) No 258/97¹ of the European Parliament and of the Council, taking into account the comments and objections of a scientific nature raised by Member States. The assessment follows the methodology set in Commission Recommendation 97/618/EC of 29 July 1997² concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council. The assessment is based on the data supplied in the original application, the initial assessment by the competent authority of Belgium, the concerns and objections of the other Member States and the responses of the applicant.

The NF is the fermented soybean extract NSK-SD®. The extract contains nattokinase, which is a serine protease composed of 275 amino acid residues. Nattokinase was originally isolated from natto, a traditional Japanese foodstuff which is produced by the fermentation of soybeans (*Glycine max* L.) with *Bacillus subtilis* var. *natto*. The soybean extract NSK-SD® is standardised to a nattokinase enzyme activity of 20,000–28,000 fibrin degradation units (FU)/g. The information provided on the composition of the NF, the specifications, batch-to-batch variability and the stability is sufficient and does not raise safety concerns.

The production process is sufficiently described and does not raise concerns about the safety of the NF.

The applicant intends to use the fermented soybean extract NSK-SD® in food supplements. The target population proposed by the applicant is healthy men and women over the age of 35 years, excluding pregnant and lactating women. The proposed maximum intake is 100 mg NSK-SD®/day (corresponding to a maximum of 2,800 FU/day).

Taking into account the composition of the NF and the intended use levels, the Panel considers that the consumption of the NF is not nutritionally disadvantageous.

The Panel notes that nattokinase exhibits *in vitro* fibrinolytic activity and *in vivo* thrombolytic activity in animals when administered parenterally. However, the information provided with respect to absorption, distribution, metabolism and excretion of the NF does not allow conclusions to be drawn on the absorption of active nattokinase or any functional metabolites therefrom.

A bacterial reverse mutation test did not show any indication of mutagenicity. The NF was not clastogenic in an *in vitro* chromosome aberration assay.

In a subchronic 90-day oral toxicity study, NSK-SD® (enzyme activity 21,900 FU/g) was administered to Sprague–Dawley rats at doses of 0, 100, 300 or 1,000 mg/kg body weight (bw) per day. Based on the observations from this study (including the absence of statistically significant findings in haematological parameters), the Panel considers that the no observed adverse effect level (NOAEL) is 1,000 mg/kg bw per day, the highest dose tested.

A number of unpublished and published human studies were provided, which were carried out in both healthy and diseased subjects including people on anticoagulants. Owing to the low power of the studies and inconsistencies in results between and within the studies, the Panel considers that the evidence provided in the human studies is insufficient to conclude on the safety of the NF.

Information was provided on the microbiological status of the NF. The Panel considers that the information provided does not raise safety concerns.

With regard to allergenicity, the Panel considers that the risk of allergic reactions to the NF is not dissimilar to that associated with other soy-derived products.

¹ Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients (OJ L 43, 14.2.1997, p. 1). <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1997R0258:20090120:EN:PDF>

² 97/618/EC: Commission Recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council (OJ L 253, 16.9.1997, p. 1–36). http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.1997.253.01.0001.01.ENG

Taking into account the NOAEL of 1,000 mg/kg bw per day in the subchronic toxicity study in rats, and considering the proposed maximum intake level for the NF of 100 mg NSK-SD®/day (corresponding to 1.43 mg/kg bw per day for a 70-kg person), the Panel concludes that the margin of exposure (of 700) is sufficient.

The Panel concludes that the NF, the fermented soybean extract NSK-SD®, is safe under the intended conditions of use as specified by the applicant.

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

On 8 May 2014, the company Japan Bio Science Laboratory (JBSL) submitted a request under Article 4 of the Novel Food Regulation (EC) No 258/97³ to place on the market a fermented soybean extract (Nattokinase NSK-SD®) as a novel food (NF).

On 1 December 2014, the competent authority of Belgium forwarded to the Commission its initial assessment report, which came to the conclusion that the fermented soybean extract (Nattokinase NSK-SD®) meets the criteria for acceptance of a NF defined in Article 3(1) of Regulation (EC) No 258/79.

On 6 January 2015, the Commission forwarded the initial assessment report to the other Member States (MS). Several of the MS submitted comments or raised objections.

The concerns of a scientific nature raised by the MS can be summarised as follows:

- Some MS commented that no data have been provided on the long-term safety of the NF. MS expressed concerns that the product might have effects on haematological parameters, in particular on blood coagulation. One case report (Chang et al., 2008) was identified, in which a patient on antihypertensive agents and low-dose aspirin for secondary stroke prevention experienced an acute cerebellar haemorrhage and cerebral microbleeds after concomitant consumption of nattokinase.
- Some MS requested further details on the composition of NSK-SD®, in particular with regard to the starch carrier and the protein fraction in NSK-SD® and their potential to elicit allergic reactions.
- Some MS commented that no evidence was provided to substantiate whether the nattokinase consumed orally was absorbed into the blood stream.
- One MS expressed concerns with regard to potential adverse effects of the nattokinase on mucous membranes.

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002⁴, the European Food Safety Authority (EFSA) is asked to carry out the additional assessment for the fermented soybean extract NSK-SD® as a NF in the context of Regulation (EC) No 258/97.

EFSA is asked to carry out the additional assessment and to consider the elements of a scientific nature in the comments raised by the other MS.

2. Data and methodologies

2.1. Data

The assessment of the safety of this NF is based on data supplied in the original application, the initial assessment by the competent authority of Belgium, the concerns and objections of the other MS and the responses of the applicant (See 'Documentation provided to EFSA').

In accordance with Commission Recommendation 97/618/EC,⁵ the fermented soybean extract NSK-SD® is allocated to Class 2.1, i.e. the source of the NF has a history of food use in the Community. The data are required to comply with the information required for NFs of Class 2.1, i.e. structured schemes I, II, III, IX, X, XI, XII and XIII of Commission Recommendation 97/618/EC. In the current opinion, these structured schemes are listed in Sections 3.1–3.8. The intention is to use the NF in food supplement products (in capsule, tablet and powder form). This assessment concerns only risks that might be associated with consumption of the NF at the proposed conditions of use, and is not an assessment of the efficacy of the fermented soybean extract NSK-SD® with regard to any claimed benefit.

³ Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. OJ L 43, 14.2.1997, p. 1–6.

⁴ Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

⁵ Commission Recommendation 97/618/EC: Commission Recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council. OJ L 253, 16.9.1997, p. 1–36.

2.2. Methodologies

The assessment follows the methodology set out in Commission Recommendation 97/618/EC of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council.

3. Assessment

3.1. Specification of the NF

The NF is a fermented soybean extract with the trade name of NSK-SD®. It contains a proprietary nattokinase isolated from natto, a foodstuff produced by the fermentation of (non-genetically modified) soybeans (*Glycine max* L.) with a selected strain of *Bacillus subtilis* var. *natto*. The fermented soybean extract NSK-SD® is standardised to have a nattokinase enzyme activity of 20,000–28,000 fibrin degradation units (FU)/g as assessed by an assay described by Takaoka et al. (2010). One unit (1 FU) is defined as the amount of enzyme that increases the absorbance (by fibrinogen degradation products) of the sample at 275 nm by 0.01/min under the conditions as specified in a spectrophotometry assay (Takaoka et al., 2010).

The nattokinase is a 27.728 kDa serine proteinase classified as a member of the subtilisin family and scientifically identified as Subtilisin NAT.NK. It is composed of 275 amino acid residues (Fujita et al., 1993). The amino acid sequence has been provided by the applicant. The nucleotide sequence of the nattokinase (Subtilisin NAT) gene exhibits a high homology with other subtilisin enzymes and is 99.5% homologous to subtilisin E and 99.3% homologous to subtilisin amylosaccharitus (Nakamura et al., 1992). The applicant establishes the identity of the nattokinase in NSK-SD® by DNA sequencing of NSK-SD®-producing bacteria and comparing the sequence to published data (Nakamura et al., 1992). The maximum fibrinolytic activity occurs around 65°C at a pH of 10.5.

NSK-SD® is an odourless milk-white coloured powder. It is comprised of 30% fermented soybean extract powder and 70% resistant dextrin (as carrier) from corn starch, which is added during the processing. Vitamin K₂ is removed during the manufacturing process.

Table 1: Specifications of NSK-SD®

Parameter	Specification	Method
Nattokinase activity	20,000–28,000 FU/g	Assay method of nattokinase ^(a)
Identity	Confirmable	IBox method ^(b) , HPLC
Condition	No offensive taste or smell	Sensory test
Loss on drying	≤ 10%	1 g, 105°C, 4 h
Vitamin K ₂	≤ 0.1 ppm	HPLC method
Contaminants		
Heavy metals	≤ 20 ppm	ICP/MS method
Lead	≤ 5 ppm	ICP/MS method
Arsenic	≤ 3 ppm	ICP/MS method
Microbiological specifications		
Total viable aerobic count	≤ 1,000 CFU/g	U.S. FDA BAM (Chapter 3)
Yeast and mould	≤ 100 CFU/g	U.S. FDA BAM (Chapter 18)
Coliforms	≤ 30 CFU/g	ISO 4832:2006
Spore-forming bacteria	≤ 10 CFU/g ^(c)	Soybean–Casein Digest (SCD) Agar method
<i>Escherichia coli</i>	Negative/25 g	US FDA BAM (Chapter 4a)
<i>Salmonella</i> sp.	Negative/25 g	AOAC sec. 967.26
<i>Listeria</i>	Negative/25 g	AOAC 960701

AOAC: Association of analytical communities; BAM: bacteriological analytical manual; CFU: colony forming units; FDA: US Food and Drug Administration; FU: fibrin degradation unit; HPLC: high-performance liquid chromatography; ICP/MS: inductively coupled plasma mass spectrometry; ISO: International Association for Standardization.

(a): As described by Takaoka et al. (2010).

(b): Analysis of the degradation pattern of oxidised insulin chain B by nattokinase employing HPLC (JBLS).

(c): Limit of detection.

Proximate analyses have been provided for five batches of NSK-SD®. The product consists primarily of carbohydrates (> 90% (by calculation)), total protein (ca. 5.5% (Kjeldahl)), moisture (ca. 3% (vacuum oven method)), ash (ca. 0.3% (dry ash method)) and fat (0.1% (Soxhlet)). The percentage of the carbohydrate content was calculated as 100-(moisture + protein + fat + ash).

The applicant indicated that the contribution of the nattokinase to the total protein fraction is about 89–93%, as derived from calculation considering a purified (< 99%) nattokinase activity of 480 FU/mg. According to the applicant, the remaining protein fraction (other than nattokinase) comprises peptides and amino acids-containing fragments that do not induce an immune response to soy-protein or nattokinase-specific antibodies. To support this statement, the applicant submitted the results of an enzyme-linked immunosorbent assay (ELISA) applied to two batches, where the content of soybean protein was below the limit of detection (i.e. 1 µg/g).

NSK-SD® has been analysed for potential bioactive compounds and none of the following have been detected (unless otherwise specified in brackets): adenine, xanthine, guanine, hypoxanthine, daidzin (0.5 mg/100 g), daidzein, genistin (1.2 mg/100 g), genistein and tyramine (< 0.5 mg/100 g).

Batch-to-batch analyses (Table 2) were provided for five different batches of NSK-SD® in order to confirm that the manufacturing process is reproducible and adequate to produce a product that is within the specifications as set above.

Table 2: Analyses of five batches of NSK-SD®

Parameter	Specification	Batch results				
		RWVF1 5/9/2012	SQXB1 21/3/2013	STXI1 28/6/2013	SWVG1 6/9/2013	SZVJ1 9/12/2013
Nattokinase activity	20,000–28,000 FU/g	23,700	25,300	24,600	24,800	24,100
Identity	Confirmable	Conforms	Conforms	Conforms	Conforms	Conforms
Condition	No offensive taste or smell	Conforms	Conforms	Conforms	Conforms	Conforms
Loss on drying	≤ 10%	3.80	3.10	2.80	2.90	3.40
Vitamin K2	≤ 0.1 ppm	nd	nd	nd	nd	nd
Contaminants						
Heavy metals	≤ 20 ppm	0.117	0.009	0.009	0.009	0.009
Lead	≤ 5 ppm	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01
Arsenic	≤ 3 ppm	≤ 0.1	≤ 0.1	≤ 0.1	≤ 0.1	≤ 0.1
Microbiological specifications^(a)						
Total viable aerobic count (CFU/g)	≤ 1,000 CFU/g	≤ 300	≤ 300	≤ 300	≤ 300	≤ 300
Yeast and mould (CFU/g)	≤ 100 CFU/g	≤ 10	≤ 10	≤ 10	≤ 10	≤ 10
Coliforms (CFU/g)	≤ 30 CFU/g	≤ 10	≤ 10	≤ 10	≤ 10	≤ 10
<i>Escherichia coli</i>	Negative/25 g	Negative	Negative	Negative	Negative	Negative
<i>Salmonella</i> sp.	Negative/25 g	Negative	Negative	Negative	Negative	Negative
<i>Listeria</i>	Negative/25 g	Negative	Negative	Negative	Negative	Negative

CFU: colony forming units; FU: fibrin degradation unit; nd: not detected.

(a): For three additional batches, the applicant provided results on the amount of spore-forming bacteria (including *Bacillus subtilis*) which were below the limit of detection (i.e. ≤ 10 CFU/g).

The Panel considers that the information provided on the composition, the specifications and the batch-to-batch variability of the NF is sufficient and does not raise safety concerns.

3.1.1. Stability of the NF

Nattokinase has been shown to be stable under various pH and temperature conditions (Sumi et al., 1987). NSK-SD® (packaged in aluminium bags) was confirmed to be stable over an 18 month period under temperature conditions of 40°C based on its nattokinase activity. At room temperature, NSK-SD® was shown to be stable for 42 months. In a 25% aqueous solution at a pH of 5.5–10 and at a temperature of 25°C, NSK-SD® was shown to be stable for 24 h, but it was labile below pH 5.0.

The Panel considers that the data provided sufficient information with respect to the stability of the NF.

3.2. Effect of the production process applied to the NF

Soybean powder is fermented at 37°C using a strain of *B. subtilis* var. *natto*, with the addition of corn starch, soybean oil, calcium carbonate and water. Thereafter, nattokinase is isolated by performing several filtration steps. The product is standardised to an enzyme activity of at least 20,000 FU/g and a maximum of 28,000 FU/g using a suitable carrier (i.e. resistant dextrin). Vitamin K₂ is removed during the manufacturing process.

The Panel considers that the production process is sufficiently described and does not raise concerns about the safety of the NF.

3.3. History of the organism used as a source of the NF

B. subtilis var. *natto* is a subspecies of *B. subtilis*, a Gram-positive, catalase-positive bacterium commonly found in soils. *B. subtilis* was granted Qualified Presumption of Safety (QPS) status with qualification (EFSA, 2007; EFSA BIOHAZ Panel, 2015).

B. subtilis var. *natto* has a long history of consumption in Japan. It is used in the commercial production of natto, which is a traditional Japanese food produced by the fermentation of soybeans (*G. max* L.).

3.4. Anticipated intake/extent of use of the NF

The applicant intends to use Nattokinase NSK-SD® in food supplements (as soft-gel capsules, hard-shell capsules, tablets and powder). The target population for the NF is healthy men and women over the age of 35, excluding pregnant and lactating women.

The proposed maximum intake is 100 mg NSK-SD®/day, which corresponds to a maximum of 2,800 FU/day (see specifications).

3.5. Information from previous exposure to the NF or its source

Natto, i.e. boiled or steamed soybeans fermented by *B. subtilis* var. *natto*, is a traditional food in Japan. One portion (50 g) of commercially available natto typically has a nattokinase activity of 1,400–2,000 FU/pack (Takaoka, 2000; Takaoka et al., 2010), with levels ranging from 600 to 3,290 FU/pack (Takaoka et al., 2010).

Nattokinase NSK-SD® has been produced since 1996. Sales figures for Japan and the USA have been provided.

3.6. Nutritional information on the NF

Taking into account the composition of the NF and the intended use levels, the Panel considers that the consumption of the NF is not nutritionally disadvantageous.

3.7. Microbiological information on the NF

Microbiological specifications are presented in Table 1 (Section 3.1). The applicant provided analytical results for five batches which were compliant with the microbiological specifications (Table 2, Section 3.1). For three additional batches, the applicant provided results on the amount of spore-forming bacteria, including *B. subtilis*. The amount of such bacteria in the product was shown (using the SCD Agar method) to be below the limit of detection, i.e. ≤ 10 CFU/g.

The applicant provided a study (Isowa, 2003) in which culture broth containing the *B. subtilis* *natto* strain used for the production of NSK-SD® was administered to ICR-strain SPF mice (5/sex per group) in a single oral inoculation of 0 (control group) or 7.55×10^8 CFU/body. No mortalities were reported nor were any remarkable changes observed on vital signs or body weight (bw) for a period of 14 days. On day 14, animals were sacrificed, and autopsy and histopathology were carried out. There were no differences between the control group and the group which had been inoculated with *B. subtilis* *natto*.

The Panel considers that the microbiological information provided does not raise safety concerns.

3.8. Toxicological information on the NF

The fibrinolytic activity of nattokinase was first identified *in vitro* using fibrin plates (Sumi et al., 1987).

In a clot lysis assay, nattokinase was shown to be able to cleave cross-linked fibrin (Fujita et al., 1995b). Nattokinase was also shown to raise *in vitro* the activity of tissue-type plasminogen activator (tPA) (Yatagai et al., 2008). Moreover, nattokinase was reported to cleave and inactivate plasminogen activator inhibitor-1 (PAI-1) *in vitro* (Urano et al., 2001). In another *in vitro* study (Pais et al., 2006), a significant dose-dependent decrease in red blood cell aggregation and low-shear viscosity was found in blood samples upon incubation with nattokinase.

Several animal studies reported on an effect of nattokinase on thrombolysis *in vivo* (Sumi et al., 1990; Fujita et al., 1995c; Kamiya et al., 2010; Xu et al., 2014).

The Panel notes that nattokinase has *in vitro* fibrinolytic activity and *in vivo* thrombolytic activity when administered parenterally.

3.8.1. Absorption, distribution, metabolism and excretion

The applicant tested soft gel capsules 'Natural Super Kinase II' (NSK-II) containing NSK-SD® *in vitro* under acidic conditions (pH 2.0, mimicking gastric fluid) in order to get information on the activity of nattokinase when passing through the stomach. The results of this assay were provided to EFSA.

One publication (Fujita et al., 1995a) studying the fate of nattokinase in the intestine of male Wistar rats was provided. After a 24-h fasting, the animals (n = 20) were anaesthetised and a loop of intestine was extracted through an abdominal incision. A 15 cm long segment of the intestine including parts of the duodenum and jejunum was tightened at both ends and nattokinase (80 mg/kg) was injected into this part of the intestine. Before and 0.5, 1, 3 and 5 h after injection of the enzyme, blood samples were collected from the left femoral vein. There was no control group with animals being submitted to the same intervention (i.e. with placebo instead of enzyme). The fibrinogen in the plasma was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under non-reducing conditions and western blotting (using anti-fibrinogen γ -chain antibody). According to the authors, fibrinogen degradation products were detected in all rats, but breakdown patterns differed. In 14 out of the 20 rats, a 270 kDa fragment appeared within 3–5 h without further degradation of the fragment, while in the remaining six rats the 270 kDa fragment appeared within 0.5 h which was then to a low degree degraded to a 105 kDa fragment via a 200 kDa fragment. In parallel with this fibrinogen degradation, the plasma recalcification time was prolonged in these six rats at 3 and at 5 h after administration (mean \pm SEM coagulation time; before: 2.1 ± 0.5 min, 0.5 h: 2.3 ± 0.6 min, 1 h: 2.5 ± 0.6 , 3 h: 6.3 ± 2.0 , 5 h: <10 min). Nattokinase was detected in blood samples by western blotting using anti-nattokinase antibody, but no information on the number of samples analysed was provided. Besides a protein band of 28 kDa in the same position as nattokinase (MW 27.7 kDa), three further protein bands (i.e. 48, 42 and 37 kDa) were detected. These bands were also recognised by anti-fibrinogen antibodies. The nature of these bands is unclear as information on the specificity of the antibodies is lacking. The Panel therefore considers that no conclusions can be drawn from this study on the absorption of active nattokinase in rats.

In a pilot study in humans (Ero et al., 2013), 11 healthy participants (six females, five males, aged 21–65 years) ingested a single dose of 100 mg nattokinase in softgel form (NSK-SD) containing 2,000 FU. Participants were eligible for the study if they were not taking nattokinase and did not consume large amounts of natto or soy protein in their diets, if they were not using prescription medication and were free of aspirin, non-steroidal, anti-inflammatory drugs, antihistamines, fish oils and other agents with antiplatelet or anticoagulant properties. Blood samples were collected at baseline and 2, 4, 8, 12, 24 and 48 h after consumption of the nattokinase. A pharmacokinetic pattern was observed between baseline and 48 h post-dose for nattokinase or its metabolites as measured by an ELISA using a rabbit, polyclonal, anti-nattokinase antibody. Increases from baseline were shown to be statistically significant in the samples at 2, 4, 8, 12 and 24 h post-dose. However, the Panel notes the limitations of this study such as the absence of a control group and the use of a non-validated ELISA. The Panel also notes that the study did not assess the biological activity of nattokinase in blood.

The Panel considers that the available information does not allow conclusions to be drawn on the absorption of active nattokinase or its metabolites.

3.8.2. Genotoxicity

A bacterial reverse mutation test using *Salmonella* Typhimurium strains TA 98, TA 100, TA 1535 and TA 1537 and the *Escherichia coli* strain WP2 uvrA was carried out in compliance with the Japanese Standard for the testing of drugs and GLP (Fuji Biomedix, 2003a). The test substance 'Nattokinase DS' (activity 20,000 FU/g) was dissolved in distilled water and tested at doses up to 5,000 µg/plate. There was no indication of a mutagenic response in any of the strains tested, neither in the presence nor in the absence of metabolic activation system (S9 mix).

An *in vitro* chromosome aberration assay using Chinese hamster lung cells (CHL/IU) was conducted in accordance with the Japanese Standard for the testing of drugs and GLP (Fuji Biomedix, 2003b). The test substance 'Nattokinase DS' (activity 20,000 FU/g) dissolved in culture medium induced inhibition of cell growth at concentrations of 0.156 mg/mL and above. Based on the results of cell growth inhibition tests in the main study, mitotic cells were scored at three dose levels as follows: 78, 110 and 156 µg/mL for the short-term (6-h) treatment without S9 mix (cell proliferation rates were 86.4%, 105.6% and 68.4%, respectively), 55, 78 and 110 µg/mL for the short-term treatment with S9 mix (cell proliferation rates were 93.0%, 82.1% and 102.0%, respectively), and 55, 78 and 110 µg/mL for the 25-h continuous treatment without S9 mix (cell proliferation rates were approximately 105% at all three dose levels). No dose-related increases in the number of cells with structural or numerical chromosome aberrations were observed.

Even if a micronucleus assay, as recommended in the EFSA Scientific Opinion on genotoxicity testing strategies (EFSA Scientific Committee, 2011), has not been provided, the Panel considers that given the nature of the NF no safety concerns are raised.

3.8.3. Acute toxicity studies

In an acute oral toxicity study (BILIS, 1999) carried out in accordance with the Japanese guidelines for the testing of drugs, nattokinase (Biozyme NSK-FD, activity approximately 10,000 FU/g) suspended in distilled water was administered to Sprague–Dawley rats (5/sex per group) at a single dose of 2,000 mg/kg bw. Apart from diarrhoea or soft stool observed on the first day after dosing, there were no indications of adverse effects.

In a more recent study (Bozo Research Center Inc., 2006), nattokinase (NSK-SD, activity 24,700 FU/g) administered orally at doses of 1,000 and 2,000 mg/kg bw to Sprague–Dawley rats (6/sex per group) induced no adverse effects.

3.8.4. Subacute and subchronic toxicity studies

A repeated-dose 28-day oral toxicity study (Fuji Biomedix, 2002), performed according to the Guideline for Toxicity Studies of Drugs established by the Japanese Ministry of Health and Welfare (Notification No. 24, 1989; 88, 1993, Japanese Ministry of Health and Welfare), was provided. Sprague–Dawley rats (6/sex per group) were administered by gavage 'Nattokinase DS' (Japanese name: NSK-SD; 20,000 FU/g) at levels of 0 or 167 mg/kg bw per day (equivalent to 0 or 3,340 FU/kg bw per day). The amount of nattokinase was calculated as being equivalent to 100 times the usual intake of natto (50 g/60-kg individual; nattokinase activity of fermented soybeans approximately 40 FU/g). Rats were monitored for clinical signs. Body weight and food consumption were recorded, and urinalysis and ophthalmoscopy were conducted. Blood samples were taken at the end of the treatment period for haematology and blood chemistry analysis. At necropsy, macroscopic examinations were carried out and organ weights were determined. Selected organs and tissues were prepared for histopathological examinations. According to the author, the no observed adverse effect level (NOAEL) was 167 mg/kg bw per day (the only dose tested).

A subchronic oral toxicity study (Bozo Research Center Inc., 2004) has been conducted on 'Nattokinase DS' (Japanese name: NSK-SD) in compliance with the Japanese Standard for the testing of drugs and GLP. The test material (enzyme activity of the batch used: 21,900 FU/g) was dissolved in water and administered by gavage to Sprague–Dawley rats (12/sex per group) for 90 days at doses of 0, 100, 300 or 1,000 mg/kg bw per day.

The animals were housed individually and examined for general condition at least twice daily. Body weights and feed consumption were recorded at least twice weekly. Ophthalmoscopy was conducted on all animals prior to the start of treatment and on 6/sex per group during week 13. Blood was collected from all animals prior to necropsy and examined for haematological parameters (red blood cell count (RBC), haemoglobin (Hb), haematocrit (Ht), mean corpuscular volume (MCV), mean

corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), reticulocyte percentage, thrombocyte count, white blood cell count (WBC), differential white blood cell count, prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen). Serum was examined for clinical biochemistry parameters (alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), gamma-glutamyltransferase (GGT), total cholesterol, triglycerides, phospholipids, total bilirubin, glucose, blood urea nitrogen (BUN), creatinine, Na, K, Cl, Ca, P, total protein (TP), albumin, albumin/globulin (A/G) ratio, protein fractions). Urine was collected from all animals overnight and examined for a range of endpoints.

A necropsy including macroscopic examination of tissues and weighing of organs (brain, pituitary, thyroids (including parathyroids), adrenals, thymus, spleen, heart, lungs (including bronchus), submandibular and sublingual glands, liver, kidneys, testes, prostate, seminal vesicle, ovaries and uterus) was conducted on all animals. A full range of tissues was preserved for histological examination and the high-dose and control groups were microscopically analysed.

No deaths occurred during the administration period and there were no treatment-related clinical signs. Body weight of male animals of the mid-dose group was statistically significantly lower compared with the control group from day 66 to 87 of treatment (max. 8%). Feed consumption in males of the mid-dose group was significantly lower from day 42 to 84 and occasionally also in males of the high-dose group (days 66, 70, 77 and 84). Female animals showed no statistically significant differences in body weight and feed consumption, and there were no significant differences in total body weight gain of males and females.

Ophthalmoscopy showed no abnormalities. There were no statistically significant differences in haematological parameters (including PT, aPTT, fibrinogen, thrombocyte counts). Clinical biochemistry analyses showed a significantly higher ALP activity in males of the high-dose group and females of the mid-dose group. Mid-dose females also had significantly higher total cholesterol and phospholipid concentrations and mid-dose males had a lower total bilirubin concentration as well as a lower percentage of β -globulin in protein fractions. Urinalysis showed statistically significantly lower concentrations of Na, K and Cl, and a higher creatinine concentration in high-dose females. On request of the NDA Panel, the applicant confirmed that the mean values for the urine parameters (except creatinine, for which no data were available) fell within the historical control ranges.

Organ weight determinations showed a number of statistically significant differences in absolute and/or relative weights. Most of them were observed in the mid- or low-dose group and, in the absence of a dose relationship, are not considered treatment related. The only significant differences observed in mid- and high-dose males were lower absolute but not relative pituitary and heart weights. In both cases, the changes were not dose related. High-dose females showed higher absolute and relative pituitary weights; on request, the applicant confirmed that the mean values were within the historical control ranges.

Macroscopic examinations at necropsy did not identify any changes associated with treatment. Microscopic examination of tissues showed no relevant increase in the incidence of histopathological findings in animals of the high-dose group compared with the controls.

The NDA Panel considers that the NOAEL for this study is 1,000 mg/kg bw per day (equivalent to 21,900 FU/kg bw per day), the highest dose tested.

3.8.5. Human studies

A number of MS expressed safety concerns with regard to possible effects on haematological parameters in humans.

A case report (Chang et al., 2008) was identified, in which a 52-year old woman in Taiwan who had used antihypertensive agents and low-dose aspirin (100 mg/day) for secondary stroke prevention experienced an acute cerebellar haemorrhage and cerebral microbleeds after consumption of 400 mg/day nattokinase for seven consecutive days. The Panel considers that no conclusions can be inferred from this report on a causal relationship between the intake of nattokinase and haematological effects.

The applicant provided three human studies commissioned by JBSL including healthy subjects (Kazuya et al., 2006), subjects with cardiovascular diseases taking warfarin (Ninomiya and Yamada, 2008) and stroke patients on antiplatelet treatment (Shah et al., 2004).

A randomised, double-blind, placebo-controlled study (Kazuya et al., 2006) including 31 healthy volunteers (aged 20–64 years, BMI > 18 to < 28 kg/m²) aimed to assess the safety of oral consumption of nattokinase. A total of 22 subjects (12 females) were advised to consume daily three capsules containing each 36.67 mg NSK-SD® (provided by JBSL and which has been sold in the

Japanese market as 'Natural Super Kinase II' since 2001, corresponding to 2,500 FU/day) for 4 weeks, while nine subjects (4 females) received a placebo during that time. Subjects were examined before, at the end and 2 weeks after termination of the intervention period. Measured endpoints were clinical observations, body weight, blood pressure, pulse rate, haematology, clinical chemistry and urinalysis. Changes in the results of the intervention and placebo group, respectively, before and after the intervention were assessed by paired t-test, each with sexes combined and segregated. Clinical laboratory testing included fasting blood sugar, whole protein, albumin, A/G ratio, total bilirubin, neutral fat, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), urea nitrogen, creatinine, Na, K, Cl, AST, ALT, γ -glutamyl transpeptidase (γ -GTP), ALP, uric acid (UA), PT in plasma, prothrombin activity value, PT-International Normalization Ratio (PT-INR), aPTT, remnant lipoprotein cholesterol (RLP-C), white blood cells, red blood cells, Hb, Ht, MCV, MCH, MCHC and number of thrombocytes. In females, PT-INR decreased from 1.08 to 1.06 after 4 weeks treatment and to 1.03 after 2 weeks post-treatment (normal range 0.84–1.14), while PT activity rate was increased in the female food test group at 2 weeks post-treatment (92.2%) compared with before treatment (88.6%) (normal range 80–100%). Slight but significant changes, which were within the normal range, were observed within both test and placebo group for a number of parameters tested. No changes were observed for blood pressure or pulse or urinalysis, which included pH, protein, glucose, occult blood, bilirubin and urobilinogen. Reported adverse events were comparable in both the intervention and the placebo group. Upon request by EFSA, the applicant provided between group analyses (comparing the NSK-SD® group with the placebo group) for the blood coagulation-related parameters. No significant differences were observed between the two groups at any time points for aPTT, PT, prothrombin activity value or PT-INR. Also upon request by EFSA, the applicant conducted post-hoc power analyses for the coagulation parameters using three separate approaches. The power for the coagulation parameters examined ranged from 5% to 50% (Cohen's effect size approach was indicated to have the greatest power, while the post-hoc approach had the lowest power). The Panel considers that this study does not show adverse effects on blood coagulation parameters at a daily intake of 110 mg NSK-SD® in healthy adults for 4 weeks. However, the Panel notes the very low statistical power of the study.

A double-blind, placebo-controlled study (Ninomiya and Yamada, 2008) was carried out in adults with cardiovascular diseases taking warfarin. The study was conducted for a total of 6 months in 30 adults (16 males, 14 females, 59 ± 10 years) of whom 15 took 1,700 FU of NSK-II (2 capsules/day) at breakfast and 15 took a placebo. In another 30 adults (17 males, 13 females, 59 ± 7.7 years), 15 subjects took 3,400 FU of NSK-II (4 capsules/day, 2 after breakfast, 2 after dinner) and 15 took a placebo. Several clinical symptoms were recorded as outcomes. These included shortness of breath, walking, coldness of hands and foot, and skin conditions. Various blood biochemistry analyses and coagulation–fibrinolysis analyses, such as aPTT, PT, PT-INR, D-dimer, plasmin inhibitor complex (PIC), total PAI-1, and high-sensitivity C-reactive protein (hs-CRP) were also measured. Statistical analysis was performed using t-test for (1) comparison of the change rate (%) between the prior to and each month after the intake and the placebo (and a separate comparison of those over 60 years of age); (2) low-dose subjects vs placebo, low-dose subjects over 60 vs placebo and high-dose subjects vs placebo. At some of the six measuring times, significant differences were found for aPTT, PT, D-dimer, PT-INR, t-PAI-1 and CRP, when compared to baseline or to the respective placebo value. However, the Panel noticed the inconsistency of the findings. Upon request by EFSA, the applicant conducted additional analyses on the coagulation parameters (comparing placebo with the low- and high-dose groups) using mixed-model analysis. There were no statistically significant results in any of the parameters at baseline. With regard to the between group analyses, the results were not statistically significant for any of the coagulation parameters, with the exception of PT activity value at 1, 5 and 6 months in the low-dose group which was elevated 1.25-, 1.31- and 1.44-fold at each time point relative to the control group, respectively. However, the standard deviation was relatively large. Additionally, for PIC, there was an overall significant decrease for the high-dose NSK group relative to the control ($p = 0.01$). Again, the standard deviation was high and no statistical differences were observed at each individual time point. The applicant also conducted power analyses for both comparisons, i.e. placebo vs low-dose group and placebo vs high-dose group. Using Cohen's approach, the statistical power ranged from 18% to 35% for a medium effect size and from 67% to 96% for a large effect size. The applicant commented that the study was designed as an efficacy study rather than a safety study. The Panel notes that although some differences between the study groups were observed these differences were inconsistent and not dose related. In addition, the Panel notes that this study was not designed and not sufficiently powered to demonstrate adverse effects on blood

parameters. The Panel considers that this study is insufficient to conclude on the safety of NSK in patients with cardiovascular diseases concurrently taking warfarin.

In an open-label, uncontrolled multicentre study (Shah et al., 2004) run in three hospitals in Mumbai, 12 patients with acute, mild to moderate ischaemic stroke (seven males and five females, mean age 53.3 years) were given three capsules of 50 mg NSK (corresponding to 2,000 FU) over 7 days, orally at 8 h intervals in addition to low molecular weight heparin and antiplatelet treatment (low-dose aspirin 325 mg). The primary outcome of the study was the prevention of stroke progression. Safety was evaluated by subjective and objective symptoms, vital signs, haematologic test and biological examination. The congealing fibrinogenolysis system was examined on the day of hospitalisation, second and seventh day after taking NSK as secondary endpoint. Using a Wilcoxon signed-rank test, significant changes were detected in various blood and coagulation parameters under the treatment. Adverse events, such as increase in blood lipids, AST and ALT, haematemesis, lower respiratory infection, headache, constipation and blood pressure increase, seizure due to termination of antiepilepsy drug administration and rib fracture, were reported. No urinalysis was performed. The Panel notes that this study in 12 ischaemic stroke patients on conventional treatment did not include a control group. The Panel considers that no conclusion can be drawn from this study in patients with ischaemic stroke with regard to the safety of NSK.

The applicant provided three additional studies (Sumi et al., 1990; Hsia et al., 2009; Kurosawa et al., 2015) that addressed fibrinolytic effects following consumption of nattokinase.

A double-blind, placebo-controlled, cross-over study (Kurosawa et al., 2015) was carried out in 12 healthy young men (age 22.3 ± 0.6 years, BMI 21.6 ± 0.5). Following the baseline blood draw, each subject was randomised to receive either a single dose of 2,000 FU nattokinase (NSK-SD) or placebo with subsequent cross-over of the groups after a washout of at least 2 weeks. Blood samples were collected at 2, 4, 6 and 8 h following administration of nattokinase/placebo for analysis of coagulation–fibrinolysis parameters. For the statistical analysis, two-way repeated measures analysis of variance between groups was performed. After the intake of nattokinase, significant increases were observed relative to placebo for D-dimer concentrations at 6 and 8 h (1.7 and 2.1-fold, $p < 0.05$), for antithrombin at 2 and 4 h (both by 3.6%, $p < 0.05$), for aPTT at 2 and 4 h (by 3.3% and 3.9%, $p < 0.05$) and for fibrin/fibrinogen degradation products (FDP) at 4 h (by 20.8%, $p < 0.01$), while factor VIII activity was significantly decreased at 4 and 6 h (by 8.9% and 9.8%, $p < 0.05$). All the changes, however, were within the normal range. No differences were observed in any of the other blood parameters analysed (PT-INR, fibrinogen, plasminogen, plasminogen activity, PIC, total PAI-1, factor VII activity, red and white blood cell counts, thrombocytes, protein, glucose) between nattokinase and placebo groups at any time point before and after supplementation. Through the duration of the study, no adverse events were reported by any of the subjects. The Panel notes that in this study, significant changes in coagulation parameters were observed following ingestion of the NF at the dose tested. However, the Panel notes that the observed changes were within normal ranges. The Panel considers that from this study that investigated effects of a single dose of the NF in a small number of healthy young men, no conclusion can be drawn on the overall safety of the long-term consumption of the NF.

In the open-label, uncontrolled study by Sumi et al. (1990), seven healthy Japanese volunteers ingested two capsules containing 1.3 g nattokinase (650 mg nattokinase/capsule) three times a day after meals for 8 days. There was no significant difference for the whole blood clot lysis time between the values before administration of nattokinase and the values on the eighth day. The euglobulin fibrinolytic activity increased gradually from the first to the eighth day (statistically not significant). The level of fibrin/fibrinogen degradation products in serum was significantly higher until day 4 following nattokinase administration ($p < 0.001$, $p < 0.005$), as was the amount of tissue plasminogen activator antigen on days 4 and 8 ($p < 0.05$). The Panel notes that in this study with high intake of nattokinase (3.9 g/day) no adverse effects were reported; however, the Panel considers that from this uncontrolled study in seven healthy Japanese subjects no conclusion can be drawn.

An open-label, uncontrolled clinical trial (Hsia et al., 2009) was conducted in three groups of 15 subjects each: healthy volunteers ('Healthy Group'), patients undergoing dialysis ('Dialysis Group'), and patients with any type of cardiovascular disease ('Cardiovascular Group'). Patients on anticoagulant medication (warfarin) were excluded. All subjects ingested two capsules of 400 mg nattokinase (2,000 FU/capsule) daily for 2 months. An intention-to-treat analysis was performed on all 45 enrolled subjects. By use of mixed-model analysis, a significant time effect, but not group effect, was observed in the change from baseline of fibrinogen ($p = 0.003$), factor VII ($p < 0.001$) and factor VIII ($p < 0.001$), i.e. plasma levels of the three coagulation factors continuously declined during

intake. The extent of decrease was similar between groups. After 2 months of administration, fibrinogen, factor VII, and factor VIII were decreased by 9%, 14% and 17%, respectively, for the Healthy Group; 10%, 7% and 19%, respectively, for the Dialysis Group; and 7%, 13% and 19%, respectively, for the Cardiovascular Group. The fibrinogen values for healthy and cardiovascular groups were within the reference range. While fibrinogen levels in the dialysis group were above the upper limit of the reference range at baseline, after consumption of nattokinase for 2 months, these levels had fallen within reference ranges. No notable adverse events were observed in any of the subjects. The Panel notes that in this uncontrolled study in healthy subjects and in patients, significant decreases in coagulation factors were reported. However, the Panel considers that no conclusions can be drawn from this uncontrolled study on the safety of the NF.

The Panel considers that in view of the low power of the studies and inconsistencies in results between and within the studies, the evidence provided by the human studies is insufficient to conclude on the safety of the NF.

3.9. Allergenicity

The fermented soy bean extract NSK-SD® contains materials from soybeans. NSK-SD® is comprised of approximately 5% protein. The applicant provided the results of an ELISA used on two batches of the product, where the content of soybean protein was below the limit of detection (i.e. 1 µg/g NF).

The Panel considers that the risk of allergic reactions to the NF is not dissimilar to that associated with other soy-derived products.

4. Discussion

The NF is the fermented soybean extract NSK-SD®. The extract contains a specific serine protease, nattokinase, and is standardised to an enzyme activity of 20,000–28,000 FU/g. The information provided on the composition, the specifications, batch-to-batch variability and the stability of the NF is sufficient and does not raise safety concerns. The production process is sufficiently described and does not raise concerns about the safety of the NF.

The applicant intends to use the nattokinase NSK-SD® in food supplements. The target population proposed by the applicant is healthy men and women over the age of 35 years, excluding pregnant and lactating women. The proposed maximum intake is 100 mg NSK-SD®/day (corresponding to a maximum of 2,800 FU/day).

Taking into account the composition of the NF and the intended use levels, the Panel considers that the consumption of the NF is not nutritionally disadvantageous.

The Panel notes that nattokinase exhibits *in vitro* fibrinolytic activity and *in vivo* thrombolytic activity in animals when administered parenterally. However, the information provided with respect to absorption, distribution, metabolism and excretion of the NF does not allow conclusions to be drawn on the absorption of active nattokinase or any functional metabolites therefrom.

A bacterial reverse mutation test did not show any indication of mutagenicity. The NF was not clastogenic in an *in vitro* chromosome aberration assay.

In a subchronic 90-day repeated dose oral toxicity study, NSK-SD® (enzyme activity of the batch 21,900 FU/g) was administered to Sprague–Dawley rats at doses of 0, 100, 300 or 1,000 mg/kg bw per day. Based on the observations from this study, the Panel considers that the NOAEL is 1,000 mg/kg bw per day, the highest dose tested.

A number of published and unpublished human studies were provided, which were carried out in both healthy and diseased subjects including people on anticoagulants. Owing to the low power of the studies and inconsistencies in results between and within the studies, the Panel considers that the evidence provided in the human studies is insufficient to conclude on the safety of the NF.

The Panel notes that in the 90-day rat study no statistically significant effects in haematological parameters (including PT, aPTT, fibrinogen, thrombocyte counts) were observed. Considering the NOAEL of 1,000 mg/kg bw per day, and the maximum proposed intake level for the NF of 100 mg NSK-SD®/day (corresponding to 1.43 mg/kg bw per day for a 70-kg person), the Panel concludes that the margin of exposure (of 700) is sufficient. When considering the enzyme activity of the NF used in the subchronic toxicity study, the NOAEL amounts to 21,900 FU/kg bw per day. Considering the maximum proposed intake for the NF of 100 mg/day corresponding to a maximum of 2,800 FU/day (i.e. 40 FU/kg bw per day for a 70-kg person), the Panel concludes that the margin of exposure (of 550) with regard to the enzyme activity is sufficient.

5. Conclusions

The Panel concludes that the NF, the fermented soybean extract NSK-SD®, is safe under the intended conditions of use as specified by the applicant.

Steps taken by EFSA

- 1) Letter from the European Commission to the European Food Safety Authority with the request for a scientific opinion on the safety of fermented soybean extract (Nattokinase-NSK-SD®) as a novel food. Ref. Ares(2015)1702324, dated 22 April 2015.
- 2) Dossier 'JBSL Nattokinase NF Dossier' received by EFSA on 7 May 2015, which was submitted by Japan Bio Science Laboratory (JBSL).
- 3) On 24 June 2015 and on 18 November 2015, EFSA sent requests to the applicant to provide missing/complementary information.
- 4) On 25 and 26 June 2015, on 3 and 7 July 2015 and on 5 February 2016, EFSA received the missing information as submitted by the applicant.
- 5) Initial assessment report carried out by the Food Safety Authority of Belgium: 'OPINION OF THE SUPERIOR HEALTH COUNCIL No 9205 on the Application for the authorisation of a "novel food" product "fermented soybean extract (Nattokinase – NSK-SD®)"'.
- 6) Member States' comments and objections.
- 7) Response by the applicant to the initial assessment report and the Member States' comments and objections.
- 8) Additional data were provided by the applicant on 7, 8 and 23 September 2015.

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Abbreviations

A	albumin
ALT	alanine aminotransferase
ALP	alkaline phosphatase
aPTT	activated partial thromboplastin time
AST	aspartate aminotransferase
BMI	body mass index
BUN	blood urea nitrogen
bw	body weight
CFU	colony forming units
CHL	Chinese hamster lung cells
ELISA	enzyme-linked immunosorbent assay
FDA	US Food and Drug Administration
FDP	fibrin/fibrinogen degradation products
FU	fibrin degradation units
G	globulin
GGT	gamma-glutamyltransferase
GLP	good laboratory practice
γ-GTP	γ-glutamyl transpeptidase
Hb	haemoglobin
HDL-C	high-density lipoprotein cholesterol
HPLC	high-performance liquid chromatography

hs-CRP	high-sensitivity C-reactive protein
Ht	haematocrit
ICP	inductively coupled plasma mass spectrometry
ISO	International Association for Standardization
LDH	lactate dehydrogenase
LDL-C	low-density lipoprotein cholesterol
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MS	Member State
nd	not detected
NDA	EFSA Panel on Dietetic Products, Nutrition and Allergies
NF	novel food
NOAEL	no observed adverse effect level
NSK-II	Natural Super Kinase II
PAI-1	plasminogen activator inhibitor-1
PIC	plasmin inhibitor complex
PT	prothrombin time
PT-INR	prothrombin time-International Normalization Ratio
QPS	Qualified Presumption of Safety
RBC	red blood cell count
RLP-C	remnant lipoprotein cholesterol
SCD	soybean–casein digest
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SPF	specific-pathogen free
TP	total protein
tPA	tissue-type plasminogen activator
UA	uric acid
WBC	white blood cell count